Synthesis of Novel Hyperbranched Poly(ester-amide)s Based on Neutral α-Amino Acids via "AD + CBB′" Couple-Monomer Approach

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ABSTRACT: A series of novel hyperbranched poly(ester-amide)s (HBPEAs) based on neutral α-amino acids have been synthesized via the "AD + CBB′" couple-monomer approach. The ABB′ intermediates were stoichiometrically formed through thio-Michael addition reaction because of reactivity differences between functional groups. Without any purification, *in situ* self-polycondensations of the intermediates at elevated temperature in the presence of a catalyst afforded HBPEAs with multihydroxyl end groups. The degrees of branching (DBs) of the HBPEAs were estimated to be 0.40–0.58 and 0.24–0.54 by quantitative ^{13}C NMR with two different calculation methods, respectively, depending on polymerization conditions and structure of monomers. The influences of catalyst, temperature, and intermediate structure on the polymerization process and molecular weights as well as properties of the resultant polymers were investigated. FTIR, NMR, and DEPT-135 NMR analyses revealed the branched structure of the resultant polymers. The HBPEAs possess moderately high molecular weights with broad distributions, glass transition temperatures in the range of −25.5 to 36.5 °C, and decomposition temperatures at 10% weight loss under nitrogen and air in the regions of 243.4–289.1 °C and 231.4–265.6 °C, respectively. Among them, those derived from D,L-phenylalanine display the lowest degree of branching, whereas the highest glass transition temperature and the best thermal stability. © 2010 Wiley Periodicals, Inc. J Polym Sci Part A: Polym Chem 48: 5364–5374, 2010

KEYWORDS: "AD + CBB′"; amino acid; biocompatibility; hyperbranched; poly(ester-amide)s; polyesters; step-growth polymerization; strategy; thiol-ene chemistry

INTRODUCTION Amino acids play a significant role in serving as the building blocks of proteins and intermediates in metabolism of all living things. These compounds are also low cost and efficient feedstocks especially in chiral organic synthesis and biochemistry. Importantly, amino acids have been increasingly developed as the components of a range of biodegradable and biocompatible polymers to modify their physical properties and reactivities. Generally, the resultant polymers possess linear,[1–7] grafted,[8–11] branched, or star-shaped[12–15] architecture. In recent years, significant efforts have focused on the synthesis and application of a new class of modern polymer called hyperbranched polymer (HBP) with randomly branched topology[16,17] as they exhibit many special merits,[18] such as three-dimensional globular architecture, low viscosity, high solubility, abundance of functional end groups, and internal cavities in the molecule.[19,20]

Amino acid-based HBPs, mainly hyperbranched polypeptides date back to the 1950s, when a number of authors investigated the thermal polymerization of AB_2-type amino acids such as L-aspartic acid, L-glutamic acid, and L-lysine.[21–28] Harada and Fox investigated the thermal homo- and co-polymerization of L-lysine and reported the formation of water-soluble polymer materials.[21,22] Although it was speculated that the polymers exhibited high degrees of branching and three-dimensional structure, no detailed characterization data were provided. Scholl et al. investigated the thermal polymerization of L-lysine monohydrochloride and characterized the structure of the resultant hyperbranched polymers by means of ^1H NMR spectroscopy.[29(a)] The DBs of the hyperbranched polylysines obtained were typically 0.35–0.45, which are smaller than the predicted value of 0.5.[29(a)] They further discussed the feasibility of three approaches to control polymer architecture during the thermal hyperbranched polymerization of L-lysine hydrochloride.[29(b)] Menz and Chapman explored the synthesis of hyperbranched polylysine using the *N*-hydroxysuccinimide ester of L-lysine dihydrochloride as the AB_2 building block. The DBs of these polymers ranged from 0.32 to 0.64.[30] Rohlfing reported the thermal copolymerization of aspartic acid and glutamic acid at less than 100 °C. Although the preparations have not been

Additional Supporting Information may be found in the online version of this article. Correspondence to: Y.-S. Li (E-mail: ysli@ciac.jl.cn)

characterized in detail, one demonstrated property of the polymer is the formation of microsphere.[27] Recently, Pan and coworkers have successfully prepared well-defined hyperbranched polystyrenes by polymerization of AB_2 macromonomer derived from L-aspartic acid.[31] However, most of the amino acid-based HBPs were prepared from acidic and (or) basic amino acids, typically glutamic acid, aspartic acid, and lysine, whereas those based on neutral amino acids are rare, except some examples copolymerized with acidic or basic amino acids.[22,24,25] Recently, a series of novel hyperbranched poly(ester-amide)s (HBPEAs) from neutral amino acid and gallic acid were reported by our group.[32] The hydrolytic and enzymatic degradation studies indicated that the HBPEAs were degradable hydrolytically as well as enzymatically, and the rate of hydrolytic degradation increases with the pH value of the solution.[32] The attractive properties of the degradable polymers promote us to further synthesize other hyperbranched polymers from neutral amino acids.

In this article, we reported the synthesis of another class of HBPEAs derived from neutral α-amino acids via a technique termed as "AD + CBB′," which is based on nonequal reactivities of different functional groups. The rapid reaction between C and D groups resulted in the predominant formation of an ABB′ intermediate, which was subjected to *in situ* self-polycondensation in the presence of a catalyst to obtain hyperbranched macromolecules. Influences of catalyst, temperature, and steric effect of the intermediate on the polymerization and properties of resultant polymers were investigated. The hyperbranched structure of the resulting polymers was verified by FTIR in combination with ^{1}H NMR, ^{13}C NMR, and DEPT-135 NMR techniques.

EXPERIMENTAL

General Procedures and Materials

Glycine, D,L-alanine, D,L-2-aminobutyric acid, and D,L-phenylalanine were purchased from ACROS and used as received. 1-Thioglycerol (90 wt % aqueous solution) was purchased from Aladdin and used without any purification. Acryloyl chloride was used as received. CH_2Cl_2 was purified by a solvent purification system (MBraun, Germany). Triethylamine (Et_3N) was distilled over CaH_2 after refluxing for 12 h. Other organic reagents and solvents were analytically pure and used without purification.

^{1}H NMR spectra were recorded on a Bruker AV 300 MHz spectrometer, ^{13}C NMR spectra were recorded using a Varian Unity 400 spectrometer operating at 100.0 MHz with D_2O or DMSO-d_6 as the solvent, the residual 1H solvent peak as reference and the solvent carbon signal as the standard, respectively. FTIR spectra were recorded on a Bio-Rad FTS-135 spectrophotometer. Glass transition temperature (T_g) was measured by differential scanning calorimetry (DSC) (TA Instruments DSC Q20) under nitrogen atmosphere with the heating rate of 10 °C/min from −50 to 100 °C, The specimens were crimp sealed in aluminum crucibles. The T_g was taken as the midpoint of the inflection tangent, upon the second heating scan. Thermogravimetric analysis (TGA) was conducted on a Perkin-Elmer Pyris 1 thermogravimetric analyzer. The sample was heated from 40.0 to 900.0 °C with a heating rate of 10 °C/min under nitrogen and air. HPLC were obtained on a Waters 1525 binary HPLC pump and 2487 dual λ absorbance detector with a Symmetry C_{18} 5 μm 4.6 × 150 mm column as immobile phase and methanol as mobile phase. ESI-MS was measured by LCQ ion trap instrument (Finnigan MAT, San Jose, CA) with an electrospray source in positive or negative ion mode. Electrospray voltage was 5.0 kV, and capillary temperature was set as 260 °C. Elemental analysis was determined with an Elementar Analysensysteme GmbH VarioEL (Germany) elemental analyzer. Size exclusion chromatography (SEC) was performed with a Waters 1525 fitted with two columns (Styragel HT3 and HT4 THF 7.8 × 300 mm column) connected in series and 2414 refractive index detector with TEDIA dimethylformamide (DMF) containing 0.05 M LiBr as the mobile phase. The SEC measurements were calibrated against narrow-dispersity polystyrene standards.

Syntheses of Vinyl Monomers AD (2a–d) Containing Amino Acid Residues

First, the various amino acids were transformed to their hydrochloride salts of amino acid methyl esters (**1a–d**) by reacting with 15 equiv methanol and 2 equiv thionyl chloride at room temperature according to the literature procedure.[33] Subsequently, the acylation processes of compounds **1a–d** were accomplished by treating with acryloyl chloride in the presence of excess Et_3N to give the AD monomers (**2a–d**) according to the ref. 34.

Syntheses of ABB′ Intermediates (3a–d) by Thio-Michael Addition Reaction

In a flask, compound **2b** (1.57 g, 10 mmol), 2 mL of CH_2Cl_2, and 0.14 mL of Et_3N (10 mol % with respect to the compound **2b**) under nitrogen atmosphere were placed. The mixture was stirred for a few minutes until compound **2b** was dissolved. Then 1-thioglycerol (0.96 mL, 10 mmol) was added slowly into the flask immersed in ice-water bath, and then the solution was kept at room temperature for 4 h. Under reduced pressure, CH_2Cl_2 was removed from the reaction system to yield green-yellow viscous liquid. Intermediates **3a**, **3c**, and **3d** were prepared analogously.

3b

Purity (HPLC): 94.8%. ^{1}H NMR (300 MHz, D_2O, ppm): 1.25 (d, —CHCH_3, 3H), 2.41–2.70 (m, —SCH_2CH(OH)—, —SCH_2CH_2—, 6H), 3.42 (dd, J_1 = 6.3 Hz, J_2 = 11.4 Hz, —CHH(OH)—, 1H), 3.51 (dd, J_1 = 3.9 Hz, J_2 = 11.4 Hz, —CHH(OH)—, 1H), 3.58 (s, —OCH_3, 3H), 3.67 (m, —SCH$_2$CH—, 1H), 4.27 (q, —NHCH—, 1H). ^{13}C NMR (100 MHz, D_2O, ppm): 14.5, 26.0, 32.9, 33.8, 47.2, 51.4, 62.7, 69.1, 172.7, 173.6. ESI-MS (*m/z*): calcd. for $C_{10}H_{19}NO_5S$, 265.1; found, 190.0 ([Fragment + H]$^+$); 247.8 ([M − H_2O + H]$^+$); 265.9 ([M + H]$^+$); 288.1 ([M + Na]$^+$). Anal. Calcd. for $C_{10}H_{19}NO_5S$: C, 45.27; H, 7.17; N, 5.02; S, 12.07. Found: C, 45.39; H, 7.21; N, 5.03; S, 12.02.

3a

Purity (HPLC): 95.9%. ^{1}H NMR (300 MHz, D_2O, ppm): 2.44–2.74 (m, —SCH_2CH(OH)—, —SCH_2CH_2—, 6H), 3.44 (dd, J_1 =

TABLE 1 *In Situ* Polymerizations of Intermediate **3b** Derived from D,L-Alanine[a]

No.	Cat.	T[b] (°C)	Time[c] (h)	M_n[d] (kg/mol)	M_w[d] (kg/mol)	PDI[d]	Yield (%)	DP[e]	DB_1[f]	DB_2[g]	$[L_1]/[L_2]$[h]	T_g[i] (°C)	$T_d^{10\%}$[j] (°C)	$T'_d{}^{10\%}$[k] (°C)
P_{b1}	$Ti(OBu)_4$	155	5	15.9	18.0	1.13	83.4	19	0.40	0.24	1:0.23	−11.52	282.6	223.1
P_{b2}	$Ti(OBu)_4$	160	9	30.2	89.8	2.97	85.5	45	0.48	0.43	1:0.39	1.33	281.4	254.2
P_{b3}	$Ti(OBu)_4$	165	5	38.8	96.8	2.49	88.3	60	0.58	0.54	1:0.46	5.35	286.7	246.4
P_{b4}	$Ti(OBu)_4$	170	0.5						Gel					
P_{b5}	$SnO(Bu)_2$	165	11	30.3	75.3	2.48	87.8	52	0.43	0.39	1:0.24	−25.52	243.2	230.2
P_{b6}	Sb_2O_3	165	9	30.2	51.2	1.69	86.5	55	0.57	0.52	1:0.45	4.75	271.8	260.9
P_{b7}	$Zn(OAc)_2$	165	24	18.9	42.6	2.20	73.0	23	0.46	0.41	1:0.33	−0.29	272.6	255.7

[a] Amount of catalyst was 0.5 g per mole of ABB′ intermediate and the initial polymerization temperatures were 60 °C for 1 h, 120 °C for 2 h.
[b] The temperature in the last stage of polymerization.
[c] The time of the last stage of polymerization.
[d] The molecular weights and PDI values were measured by SEC calibrated against narrow-dispersity polystyrene standards.
[e] DP values determined by quantitative ^{13}C NMR.
[f] DB_1 were determined by formula 1 on the basis of quantitative ^{13}C NMR.
[g] DB_2 were determined by formula 2 on the basis of quantitative ^{13}C NMR.
[h] The ratios of L_1 units to L_2 units ($[L_1]/[L_2]$) in polymers were calculated by quantitative ^{13}C NMR.
[i] The glass transition temperatures were determined by DSC measurements with a heating and cooling rate of 10 °C/min in nitrogen −50 to 100 °C.
[j] The temperatures of 10% mass loss in nitrogen were determined by TGA measurements with a heating rate of 10 °C/min from 40.0 to 900.0 °C.
[k] The temperatures of 10% mass loss in air were determined by TGA measurements with a heating rate of 10 °C/min from 40.0 to 900.0 °C.

6.3, J_2 = 11.4 Hz, —C*H*H(OH)—, 1H), 3.53 (dd, J_1 = 3.9 Hz, J_2 = 11.4 Hz, —C*H*H(OH)—, 1H), 3.61 (s, —OCH_3, 3H), 3.70 (m, —SCH_2C*H*(OH)—, 1H), 3.87 (s, —NHCH_2—, 2H). ^{13}C NMR (100 MHz, D_2O, ppm): 30.1, 37.3, 38.0, 43.2, 54.7, 66.7, 73.4, 173.3, 175.3. ESI-MS (*m/z*): calcd. for $C_9H_{17}NO_5S$, 251.1; found, 234.0 ($[M - H_2O + H]^+$); 252.0 ($[M + H]^+$). Anal. Calcd. for $C_9H_{17}NO_5S$: C, 43.01; H, 6.77; N, 5.58; S, 12.74. Found: C, 42.89; H, 6.73; N, 5.58; S, 12.79.

3c

Purity (HPLC): 95.5%. ^1H NMR (300 MHz, D_2O, ppm): 0.86 (t, J = 6.9 Hz, —CH_2CH_3, 3H), 1.58–1.68 (m, —C*H*HCH_3 1H), 1.72–1.81 (m, —C*H*HCH_3, 1H), 2.48–2.77 (m, —SCH_2 CH(OH)—, —SCH_2CH_2—, 6H), 3.48 (dd, J_1 = 6.3 Hz, J_2 = 11.7 Hz, —C*H*H(OH)—, 1H), 3.57 (dd, J_1 = 3.3 Hz, J_2 = 11.7 Hz, —C*H*H(OH)—, 1H), 3.65 (s, —OCH_3, 3H), 3.71–3.77 (m, —SCH_2C*H*(OH)—, 1H), 4.19–4.23 (m, —C*H*CH_2CH_3, 1H). ^{13}C NMR (100 MHz, D_2O, ppm): 9.6, 24.1, 27.7, 34.5, 35.4, 52.8, 54.4, 64.3, 70.7, 173.6, 174.4, ESI-MS (*m/z*): calcd. for $C_{11}H_{21}NO_5S$, 279.1; found, 204.0 ([Fragment + H]$^+$); 261.6 ($[M - H_2O + H]^+$); 279.9 ($[M + H]^+$). Anal. Calcd. for $C_{11}H_{21}NO_5S$: C, 47.29; H, 7.52; N, 5.02; S, 11.46. Found: C, 47.16; H, 7.56; N, 5.04; S, 11.41.

3d

Purity (HPLC): 96.4%. ^1H NMR (300 MHz, D_2O, ppm): 2.41–2.76 (m, SCH_2CH(OH), SCH_2CH_2, 6H), 3.03 (m, —C*H*HC$_6H_5$, 1H), 3.12 (m, —C*H*HC$_6H_5$, 1H), 3.48–3.74 (m, —SCH_2 C*H*(OH)—, —OCH_3, —CH_2(OH)—, 6H), 4.76–4.83 (m, —C*H*CH_2 C$_6H_5$, 1H), 7.06–7.25 (m, —C$_6H_5$, 5H). ^{13}C NMR (100 MHz, D_2O, ppm): 28.3, 35.7, 36.7, 38.1, 52.7, 53.8, 65.6, 71.5, 127.4, 128.9, 129.6, 136.3, 171.8, 172.6, ESI-MS (*m/z*): calcd. for $C_{16}H_{23}NO_5S$, 341.1; found, 266.0 ([Fragment + H]$^+$); 323.7 ($[M - H_2O + H]^+$); 341.9 ($[M + H]^+$). Anal. Calcd. for $C_{16}H_{23}NO_5S$: C, 56.28; H, 6.74; N, 4.10; S, 9.38. Found: C, 56.16; H, 6.74; N, 4.15; S, 9.39.

In Situ Polymerization

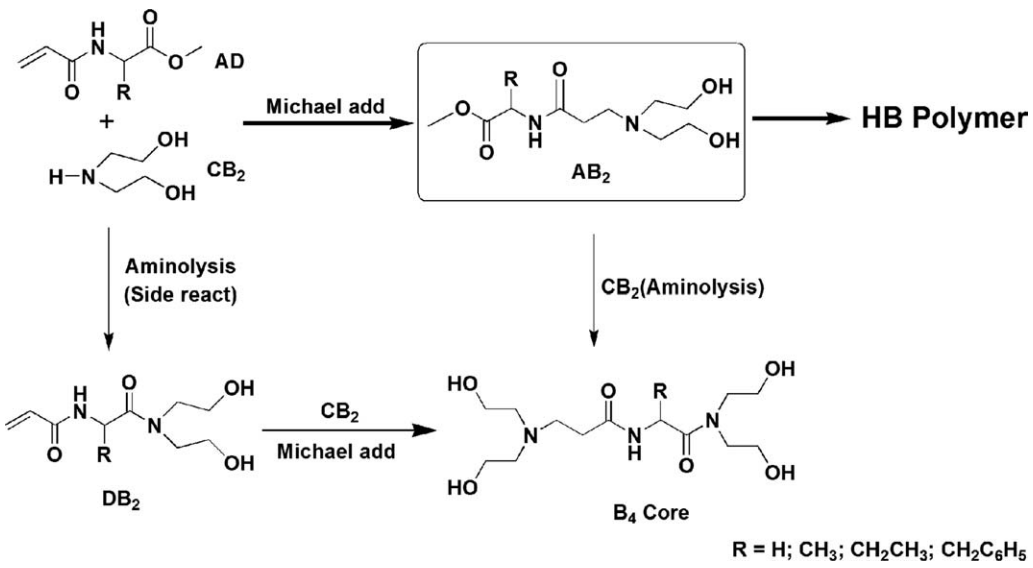
The typical polymerization of intermediate **3b** (**P_{b3}** in Table 1) is as follows. The preparation of intermediate **3b** was presented above. The CH_2Cl_2 and Et_3N was removed completely under vacuum and without further purification and isolation, 0.005 g of $Ti(OBu)_4$ was added into the flask containing the intermediate (ca. 10 mmol). Under vigorous mechanical stirring and reduced pressure, the reaction mixture was kept at 60 °C for 1 h, 120 °C for 2 h, then 165 °C until the viscosity of the system increased suddenly. Finally, the crude product was dissolved in DMF, and then poured into 200 mL of acetone. The precipitate was collected and purified by reprecipitation from DMF solution by adding excess acetone thrice. The catalyst was removed from the polymers via reprecipitation process. The resultant polymer was dried at 40 °C under vacuum for 24 h. IR (KBr, cm^{-1}): 3408 (O—H), 1739 (OC=O), 1652 (NC=O), 1545 (H—NCO). ^{13}C NMR (100 MHz, DMSO-d_6, ppm): 15.4 (*C*H_3CH—), 26.2 (—S*C*H_2CH_2CONH— in four different units), 29.3–29.9 (—*C*H_2 CONH— in four different units), 33.2–33.9 (—CH(OH)*C*H_2*S*— in four different units), 46.0 (CH_3*C*H—), 51.0 (*C*H_3O—), 59.7, 62.2, 62.9, 65.4 (—*C*H_2OH in four different units), 66.4, 69.7, 72.4, 73.0 (—*C*H(OH)— in four different units), 168.9–171.5 (—*C*ONH and —*C*OO—).

P_a

The polymerization procedure was similar to that of **P_b**. ^{13}C NMR (100 MHz, DMSO-d_6, ppm): 26.5–27.6 (—S*C*H_2CH_2 CONH— in four different units), 31.0–31.2 (—*C*H_2CONH— in four different units), 34.6–35.4 (—CH(OH)*C*H_2*S*— in four different units), 51.6 (*C*H_3O—), 61.0, 63.6, 64.4, 67.0 (—*C*H_2OH in four different units), 67.9, 70.8, 71.2, 73.6 (—*C*H(OH)— in four different units), 171.0–169.3 (—*C*ONH and —*C*OO—).

P_c

The polymerization procedure was similar to that of **P_b**. ^{13}C NMR (100 MHz, DMSO-d_6, ppm): 10.3 (*C*H_3CH$_2$—), 24.4



SCHEME 1 Possible reaction between AD monomer and diethanolamine CB_2 monomer.

(CH_3CH_2—), 27.6–27.9 (—SCH_2CH_2CONH— in four different units), 31.4–31.7 (—CH_2CONH— in four different units), 34.9–35.6 (—CH(OH)CH_2S— in four different units), 51.8 (CH_3O—), 53.4 (CH_3CH_2CH—), 61.3, 63.8, 64.6, 67.0 (—CH_2OH in four different units), 68.1, 71.4, 74.0, 74.4 (—CH(OH)— in four different units), 171.0–172.6 (—CONH and —COO—).

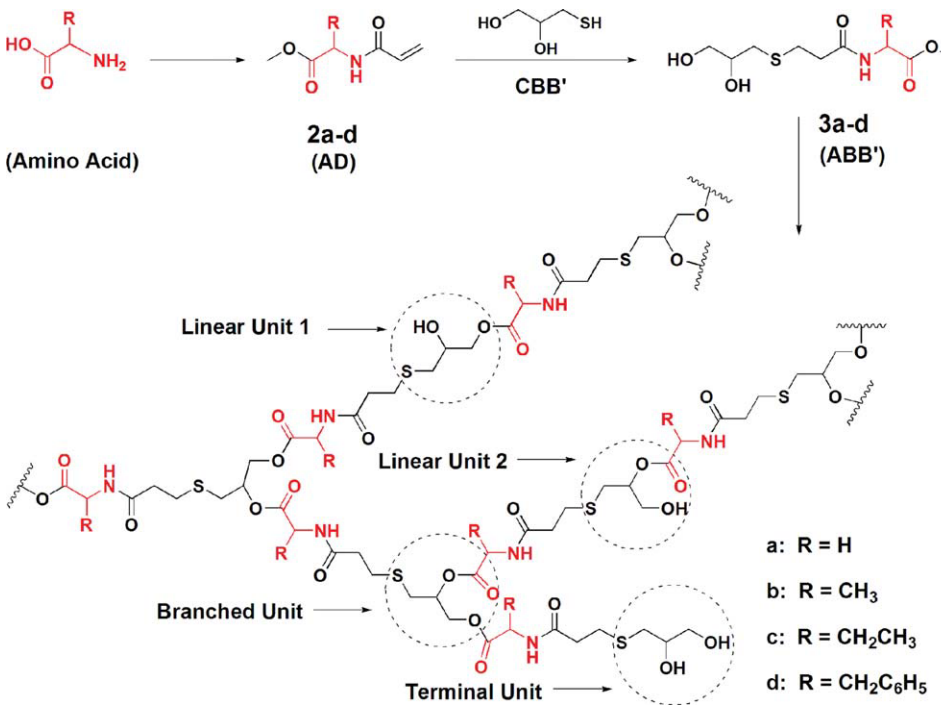
P_d

The polymerization procedure was similar to that of $\mathbf{P_b}$. ^{13}C NMR (100 MHz, DMSO-d_6, ppm): 27.3–27.7 (—SCH_2CH_2CONH— in four different units), 30.7–31.4 (—CH_2CONH— in four different units), 34.8–35.6 (—CH(OH)CH_2S— in four different units), 36.8 (Ph—CH_2—), 51.8 (CH_3O—), 53.5 (PhCH_2CH—), 61.1, 64.0, 64.5, 67.1 (—CH_2OH in four different units), 67.9, 71.3, 74.0, 74.3 (—CH(OH)— in four different units), 126.5, 128.2, 129.0, 137.1 (Ph), 170.5–172.0 (—CONH and —COO—).

RESULTS AND DISCUSSION

Molecular Design of the Approach to HBPEAs Based on Amino Acids

On the basis of the principle of "AD + CB_2" methodology, an AB_2-type intermediate will be generated due to reactivity differences between different functional groups in selected monomer pair, further *in situ* reactions among the AB_2 species result in hyperbranched macromolecules. In our study, the AB_2 intermediate was expected to be created through Michael addition reaction, which is benefited from high conversion, low probability of side reactions, mild reaction conditions, and favorable reaction rate.[35] The vinyl monomer AD containing one activated C=C double bond and one ester group, which was prepared from amino acid and acryloyl chloride, performed as the Michael acceptor. In the preliminary optimization of the Michael donor, namely, the CB_2 monomer, widely investigated dienthanolamine and diisopropanolamine were chosen due to higher reactivity of the amino group than the hydroxyl toward the unsaturated double bond of acrylamide derivative.[36] However, detailed experiments (see Supporting Information Figs. S1 and S2) showed that mixtures of complicated products were obtained instead of dominant formation of the desired AB_2 intermediate under various reaction conditions, because of existence of competitive ester aminolysis side reaction producing the DB_2 species (Scheme 1), which happened under the similar conditions to the prominent reaction of aza-Michael addition yielding the AB_2 intermediate. Furthermore, the inefficient reactivity of the nitrogen-donor may be another reason for failure. Considering that (i) mercapto group is more nucleophilic than amino group and therefore more reactive, followed by hydroxyl (SH > NH (original) > NH_2 > NH (formed) > OH);[37] (ii) mercapto group will not react with ester group under mild conditions, accordingly the side reaction occurred at the ester group can be suppressed. Therefore, the desired AB_2 intermediate can be afforded almost exclusively at the first stage. The reactions of sulfur containing compounds with alkenes were termed as thiol-ene chemistry, which are used by most authors today to describe the reactions of thiols with a wide variety of unsaturated functional groups, in addition to unactivated C—C double bond. The thiol-ene reaction can take place either by classical radical addition mechanism or Michael-type nucleophilic addition, even by a mixed mechanism. The merits, mostly resulting from the high efficiency and orthogonality of the reaction, mild reaction conditions and the compatibility of the process with sensitive functional groups and biological processes, have led to thiol-ene chemistry being used increasingly in polymer functionalization and macromolecular synthesis, as well as more traditional applications ranging from cross-linked networks to functionalized biomaterials.[38–42] In fact, many of the design features of click chemistry as proposed by Sharpless and coworkers,[43] which is widely applicable in the syntheses of dendritic and star polymers,[31,44] are also presented with the thiol-ene reaction.



SCHEME 2 "AD + CBB′" strategy for novel hyperbranched poly (ester-amide)s based on neutral α-amino acids.

Consequently, a commercially available monomer 1-thiolglycerol was finally chosen to serve as the CB_2 monomer (actually a CBB′ monomer because of different reactivities of the primary and the secondary hydroxyl groups) in our system. Scheme 2 shows the design idea. The vinyl monomer AD, an easily prepared amino acid-based compound bearing one ester group and one activated C=C double bond, with a commercially available CBB′ monomer 1-thioglycrol, thiol with one primary and one secondary hydroxyls, creates a suitable monomer pair. The amide-derivative is dominantly formed as an ABB′ intermediate with one ester and two different hydroxyl groups via the thio-Michael addition. Without further purification and isolation, the intermediate is subjected to transesterification polymerization in the presence of a catalyst to produce HBPEAs bearing numerous hydroxyl end groups.

Synthesis of ABB′ Intermediate Originated from D,L-Alanine

The use of weak base catalysts, such as Et_3N, is sufficient to catalyze the thio-Michael addition process due to readily accessible pK_a of thiol (9.66[45] for 1-thiolglycerol vs. 14.40[46] for glycerol).[35(a),47] Reaction of thiol with Et_3N results in deprotonation of the thiol to the corresponding thiolate anion and formation of the triethylammonium cation.[47,48] The thiolate, a powerful nucleophile, adds to the activated C=C bond at the electrophilic β-carbon forming a carbon-centered anion (or enolate) intermediate, which is a very strong base. Then, this anion abstracts a proton (either from the thiol or from the ammonium cation) producing the thio-Michael addition product following regioselective anti-Markovnikov's rule.[48] In this process, a relatively weak base Et_3N is used to generate a much stronger base (the carbanion or enolate) in the catalytic cycle, yielding the expected results.

The formation of the intermediate **3b** was evidenced by ^{1}H NMR at the initial stage of the reaction. Figure 1 displays the evolution of ^{1}H NMR spectra for the reaction mixture of compound **2b** and equimolar 1-thiolglycerol from 10 min to 24 h at ambient temperature in the presence of catalytic amount of Et_3N. The intermediate **3b** was formed as soon as the mixing of the two reactants and catalyst. It is clear that the peaks at 5.5–6.5 ppm ascribed to the protons of the double bond in compound **2b** gradually decreased with the prolonging of the reaction time. The new peaks emerged at higher field of 2.4–2.7 ppm which are partly overlapping, are

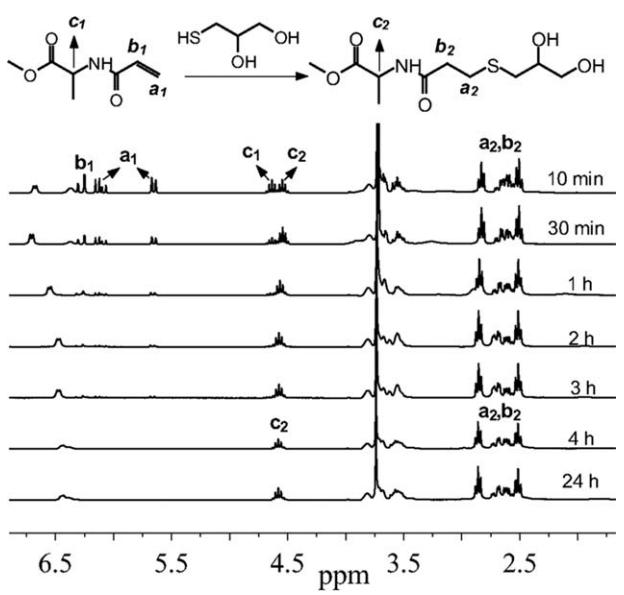


FIGURE 1 Evolution of the ^{1}H NMR spectra for the reaction system between **2b** and equimolar 1-thioglycerol over time.

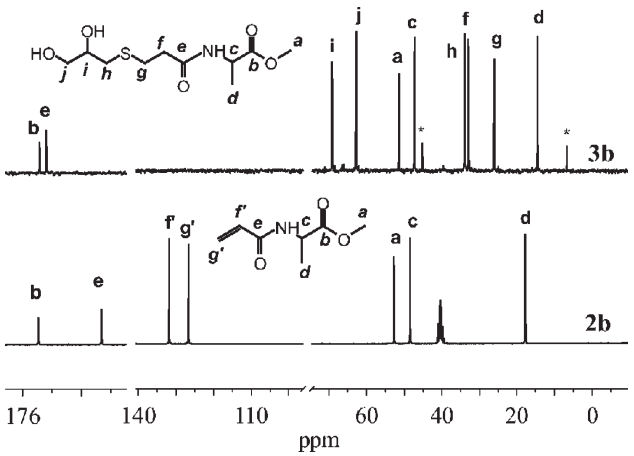


FIGURE 2 ^{13}C NMR spectra of **2b** in DMSO and intermediate **3b** in D_2O (*: Peaks originated from the protons of Et_3N).

attributed to the protons of the two methylenes formed correspondingly, indicating that the Michael addition was promoted at the β-carbon but not the α-carbon. The desired reaction was fast within the initial 1 h, and about 90% of the monomers reacted with each other. Reaction between the remaining monomers needed another 3 h, and almost no changes could be observed between the spectra recorded at 4 h and that at 24 h, which indicated that the reaction finished in 4 h, obtaining the desired molecule near quantitatively. Moreover, in this process, Michael addition of the hydroxyls to the double bond would not occur under the same conditions (see Supporting Information Fig. S3). The formation of the desired molecule was further verified by ^{13}C NMR and ESI-MS analyses of the product. As expected, ^{13}C NMR spectra (Fig. 2) proved the successful formation of the desired amide-derived intermediate. Obviously, the peaks originated from the carbons of C=C at 131.8 and 126.7 ppm exhibit a full high-yield shift to 69.1 and 62.7 ppm. ESI-MS analyses (Fig. 3) also confirmed that the Michael addition reaction was carried out in a high yield because no peak attributable to any byproducts or residuals is observed.

In Situ Self-Polycondensation to Prepare HBPEAs Derived from D,L-Alanine

The ABB′ intermediate obtained at the first stage of the reaction was directly subjected to self-polycondensation to prepare HBPEA after removal of the solvent. It is extremely important to search suitable polymerization conditions to obtain soluble HBPEA with high molecular weight. The typical results are summarized in Table 1. High vacuum was applied during the whole polymerization process till the viscosity of the polymer increased suddenly. The methanol generated was removed from the system by vacuum to break the balance to reach a high conversion of the intermediate. The results show that the temperature in the last stage of the polymerization significantly influences the molecular weights of the resultant polymers. The data in Table 1 indicate that the molecular weight rapidly increased with the increase of reaction temperature. However, crosslinking was observed at the temperature above 170 °C ($\mathbf{P_{b4}}$) probably because of ether formation and cyclic side reactions, while HBPEA with high molecular weight cannot be obtained at 150 °C even after 30 h in the presence of $Ti(OBu)_4$. In the region of 155–165 °C, the soluble polymers with high molecular weights were obtained successfully. The optimum polymerization temperature for intermediate **3b** is 165 °C.

The effect of catalysts on the polymerization was also investigated. The polymerization catalyzed by $Ti(OBu)_4$ ($\mathbf{P_{b3}}$) was faster, and higher molecular weight polymer was more easily obtained than the other catalysts used, such as $SnO(Bu)_2$ ($\mathbf{P_{b5}}$), Sb_2O_3 ($\mathbf{P_{b6}}$), and $Zn(OAc)_2$ ($\mathbf{P_{b7}}$). Part of the polymers was carbonized or oxidized (evidenced by dark color of the resultant polymers) when the later three catalysts were used due to a long time staying at high temperature to obtain polymers with high molecular weights. Therefore, $Ti(OBu)_4$ is the most efficient catalyst among several catalysts used here for the transesterification polymerization of such an intermediate.

Characterization of HBPEAs Based on D,L-Alanine

The soluble hyperbranched polymers derived from D,L-alanine were obtained under the optimal conditions, and the structure of the corresponding polymers was characterized by FTIR and NMR techniques. The FTIR spectra (see Supporting Information Fig. S4) provide evidence for the chemical structure of the polymer ($\mathbf{P_{b3}}$), showing that the characteristic absorptions of ester carbonyl and amide carbonyl groups are at 1739 and 1652 cm^{-1}, respectively, and N—H bending mode for amide group at 1545 cm^{-1}. When compared with that of the intermediate, the FTIR spectra of the polymer broaden due to the complicated repeat units and branched architecture, which are the characteristic features of HBP.[29]

Generally, there are three different units, branched units (*B*), terminal units (*T*), and linear units (*L*) in HBP prepared from a symmetric AB_2 monomer.[49] However, the intermediate formed here is actually an asymmetric AB_2 monomer, namely an ABB′ monomer, due to higher reactivity of the primary hydroxyl than the secondary hydroxyl group. Therefore, four different units exist in the resultant polymer, that is,

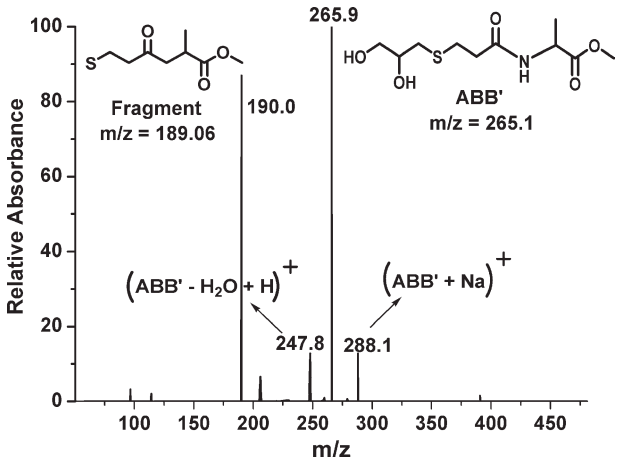


FIGURE 3 ESI mass spectrum of intermediate **3b**.

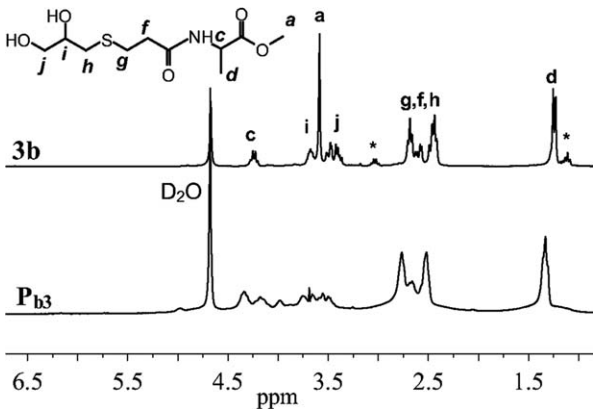


FIGURE 4 ^{1}H NMR spectra of intermediate **3b** and polymer $\mathbf{P_{b3}}$ in D_2O. The polymerization was conducted *in situ* at 165 °C for 5 h with $Ti(OBu)_4$ as the catalyst (*: Peaks originated from the protons of Et_3N).

branched units (*B*), terminal units (*T*), linear units 1 (L_1, only the primary hydroxyl group reacts), and linear units 2 (L_2, only the secondary hydroxyl group reacts). The complex structure can be confirmed by NMR spectra. The ^{1}H NMR spectra (Fig. 4) of the polymer resemble that of its monomer as their structure is alike except that the peaks in the spectra of the polymer broaden and a few new peaks in the range of 4.0–4.5 ppm are found, which indicate the complex microenvironment resulted from various architectures in the polymer. However, it is difficult to precisely assign the peaks in ^{1}H NMR spectra of the polymer because of serious overlapping of different protons. Thus, ^{13}C NMR analysis along with distortionless enhancement by polarization transfer 135 (DEPT-135) experiment (Fig. 5) was performed to further confirm different units in the polymer. Obviously, the positive resonances for methyl and methine carbons are readily distinguished from the negative resonances of methylene carbons, whereas the quaternary carbons are not found in DEPT-135 spectra. By comparing ^{13}C NMR with DEPT-135 spectra of the polymer, the four negative peaks at 65.4–59.7 ppm are correspondingly assigned to the carbon *j* (C_j in Fig. 5) of the four different units. Commonly, the exact assignments of these peaks should be based on low molecular weight model compounds, which possess structure respectively similar to the branched, terminal, and linear units in the hyperbranched polymers, to determine accurate chemical shifts of *B*, *T*, L_1, and L_2. However, the attempts to synthesize the corresponding low molecular weight compounds failed. Fortunately, Ohta and Hikino have reported that the α-carbon of the hydroxyl group will show a downfield shift, whereas β-carbon show a upfield shift when the hydroxyl is converted to acetyl functional group.[50] They further confirmed this issue in the determination of lyoniatoxin structure.[51] This theory can also be applied to our study.

First, given that the chemical shift of C_j in terminal unit is $\delta_{T,j}$, when the primary hydroxyl group is acylated to obtain linear unit 1 and the secondary hydroxyl group to linear unit 2, respectively, C_j will show downfield shift to $\delta_{L_1,j}$ ($\delta_{L_1,j} > \delta_{T,j}$) and upfield shift to $\delta_{L_2,j}$ ($\delta_{L_2,j} < \delta_{T,j}$) according to the theory mentioned above. However, it is difficult to assign C_j in branched unit ($\delta_{B,j}$) on the basis of this theory because of conflicting influences of the primary and secondary hydroxyl acylation on the chemical shift of C_j. Fortunately, the chemical shift of C_j in terminal unit should be very similar to that of the corresponding carbon in the intermediate due to their similar structure. On the other hand, typically for hyperbranched polymers, the signals of terminal units are

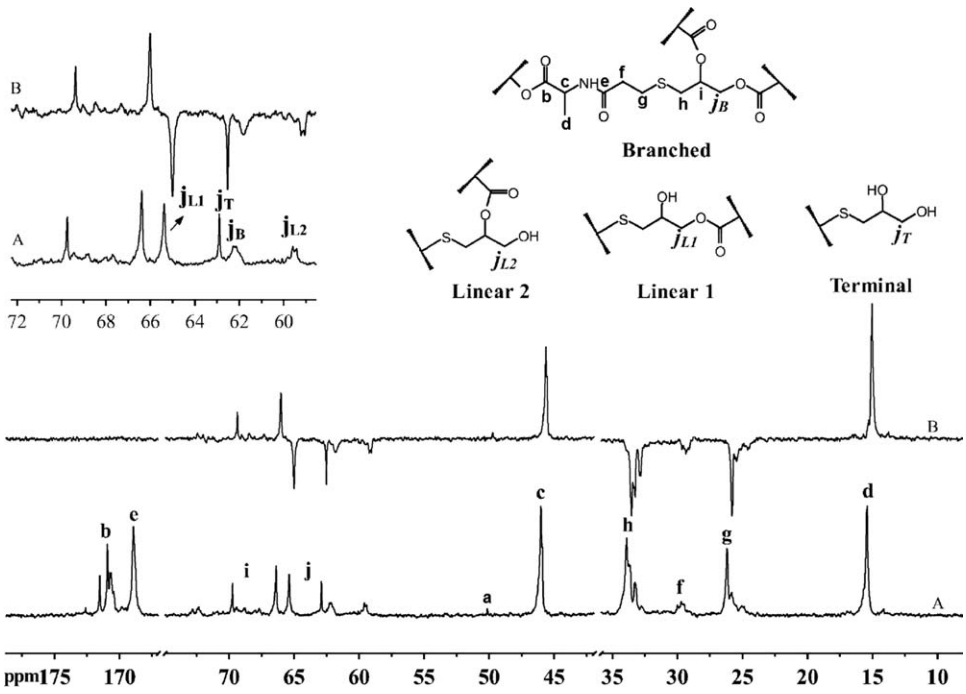


FIGURE 5 ^{13}C NMR spectra of $\mathbf{P_{b3}}$ in DMSO: $\mathbf{P_{b3}}$ (A); DEPT-135 spectrum of $\mathbf{P_{b3}}$ (B) where the CH and CH_3 resonances are above the baseline and the CH_2 resonances are directed below the baseline.

TABLE 2 *In Situ* Polymerizations of Intermediates **3a**, **3c**, and **3d** Originated from Glycine, D,L-2-Aminobutyric Acid and D,L-Phenylalanine, Respectively[a]

No.	T[b] (°C)	Time[c] (h)	M_n[d] (kg/mol)	M_w[d] (kg/mol)	PDI[d]	Yield (%)	DP[e]	DB_1[f]	DB_2[g]	$[L_1]/[L_2]$[h]	T_g[i] (°C)	$T_d^{10\%}$[j] (°C)	$T'_d{}^{10\%}$[k] (°C)
P_{b3}	165	5	38.8	96.8	2.49	88.3	60	0.58	0.54	1:0.46	5.35	286.7	246.4
P_{a1}	160	7.5	20.2	53.0	2.60	85.4	43	0.56	0.53	1:0.53	25.21	275.9	247.2
P_{a2}	165	2	17.1	55.4	3.24	88.9				u.d.			
P_{c1}	165	19	40.7	80.8	1.98	79.4	74	0.53	0.50	1:0.45	16.21	243.4	231.4
P_{c2}	175	8.5	40.0	97.0	2.42	80.7				u.d.			
P_{d1}	165	26	84.8	649.5	7.65	85.7				u.d.			
P_{d2}	175	15	53.7	252.0	4.68	89.5	75	0.41	0.33	1:0.21	36.49	289.1	265.6
P_{d3}	180	12	117.3	263.0	2.23	83.4				u.d.			

[a] The catalyst of the polymerization was $Ti(OBu)_4$ with amount of 0.5 g per mole of ABB′ intermediate and the initial polymerization temperatures were 60 °C for 1 h, and then 120 °C for 2 h.
[b] The temperature in the last stage of polymerization.
[c] The time of the last stage of polymerization.
[d] The molecular weight was measured by SEC calibrated against narrow-dispersity polystyrene standards.
[e] DP values determined by quantitative ^{13}C NMR.
[f] DB_1 were determined by formula 1 on the basis of quantitative ^{13}C NMR.
[g] DB_2 were determined by formula 2 on the basis of quantitative ^{13}C NMR.
[h] The ratios of L_1 units to L_2 units ($[L_1]/[L_2]$) in polymers were calculated by quantitative ^{13}C NMR.
[i] The glass transition temperatures were determined by DSC measurements with a heating and cooling rate of 10 °C/min in nitrogen −50 to 100 °C.
[j] The temperatures of 10% mass loss in nitrogen were determined by TGA measurements with a heating rate of 10 °C/min from 40.0 to 900.0 °C.
[k] The temperatures of 10% mass loss in air were determined by TGA measurements with a heating rate of 10 °C/min from 40.0 to 900.0 °C.

narrower than those of branched units because of their higher mobility. As a result, the peak at 62.9 ppm in the ^{13}C NMR spectra of the polymer should be assigned to C_j in terminal unit (T) by comparison with that of the intermediate at 62.7 ppm. Moreover, the peaks at 59.7, 62.2, and 65.4 ppm will be assigned to C_j in L_2, B, and L_1, respectively. Similarly, the peaks at 73.0, 72.4, 69.7, and 66.4 ppm originate from C_i of the four subunits L_2, B, T, and L_1, respectively.

The clear assignments of the four different units in the NMR spectra of the polymer are important for the determination of degree of branching (DB),[52] which is one of the most important molecular parameters of HBP, characterizing the difference in molecular structure from their linear analogue. The DBs are calculated on the basis of the terminal (T), branched (B), and linear units (L). The DB according to the definition of Hawker and Fréchet is given by[52]:

$$DB_1 = \frac{B + T}{B + T + L} \quad (1)$$

whereas a more general definition for the DB in AB_2 system is given by[53]:

$$DB_2 = \frac{2B}{2B + L} \quad (2)$$

DB_1 and DB_2 differ considerably at low molecular weights. The DB_1 possesses a value of 1 at low molecular weights and decreases with increasing molecular weight, and finally reaches a minimum value, which is the most likely explanation for DB values exceeding the predicted value of 0.5 for a random AB_2 polymerization in some literatures according to formula 1. However, the DB_2 assumes a value of 0 for low molecular weights and then slowly increases with increasing molecular weight, until it finally reaches its limiting value. Subsequently, the DB_2 increases slightly and also approaches the same plateau value as DB_1 at higher molecular weights.[54] In our study, because of severe overlapping of the polymer protons in the ^1H NMR spectra, DB values were calculated on the basis of quantitative ^{13}C NMR using the two formulas mentioned above, respectively. The DB values of the polymers prepared from intermediate **3b** were determined to be 0.43–0.58 (by Formula 1) and 0.39–0.54 (by Formula 2) except the lower one produced at relatively low temperature (155 °C) in the presence of $Ti(OBu)_4$ (**P_{b1}** in Table 1). It indicated that the molecular weights of the resultant polymers were not large enough to reach the plateau of the degree of branching referring to the results of lower values calculated by Formula 2 than Formula 1. In addition, the Tables 1 and 2 give the content ratios of linear unit 1 to linear unit 2 ($[L_1]/[L_2]$) in the polymers calculated by quantitative ^{13}C NMR. Much more contents of L_1 units in the polymers support the fact that the primary hydroxyl group exhibits greater reactivity than that of the secondary hydroxyl group. The $[L_1]/[L_2]$ decreases with increasing polymerization temperature under the same catalyst (**P_{b1}**–**P_{b3}** in Table 1), and the lower is the $[L_1]/[L_2]$, the higher is the DB value.

Because of the existence of numerous hydroxyl and amide groups, the HBPEAs obtained are highly soluble in extremely polar solvents such as methanol, DMF, and DMSO,[55] as show poor solubility in THF and $CHCl_3$. The molecular weights (M_ws) and the polydispersity indices (PDIs) of the HBPEAs were determined by SEC equipped with refractive index detector using narrow-dispersity polystyrene as standards with DMF as solvent and eluent. However, this method has only

limited suitability for HBPs. On one hand, underestimation will be caused because of their smaller hydrodynamic radii compared with their linear analogues with the same molar mass.[56] On the other hand, however, the strong interactions between the numerous polar end groups of the polymer and the solvent make the measurement results overestimate the true values. The measured data listed in Table 1 can only be used for relative comparisons. The M_n of the resultant HBPEAs ranges from 15.9 to 38.8 kg/mol and shows a strong dependence on polymerization conditions, such as different temperatures and catalysts. The PDIs estimated from SEC measurement are in the region of 1.13–2.97, which are smaller than prediction due to removal of the polymers with low molecular weights by the multistep purification process. The similar results have been reported by previous literatures.[18(a),32,36,57] Actually, the molecular weights of the polymers can be estimated on the basis of quantitative ^{13}C NMR by comparing intensity of signal (I_a) for the methylester focal group at ~51 ppm with that (I_c) of the CH signals (α-C of the amino acid residue) at ~46 ppm. According to the assignment, the DP values of the polymers were calculated by dividing the average integration values of peak c by peak a:

$$\mathrm{DP} = \frac{I_c}{I_a} + 1 \tag{3}$$

It should be noted that the calculation is based on the absence of intramolecular cyclization, thus each molecule bears a single unreacted A focal group.[58] However, slight intramolecular cyclization to ether formation may be unavoidable under such a high-polymerization temperature. The number of focal point groups, relative to repeat units, would be lower due to intramolecular cyclization, resulting in overestimation of the molecular weight evaluated with formula 3.[59] The DP values determined by quantitative ^{13}C NMR are illustrated in Table 1, which indicate that the M_n values of the polymers based on quantitative ^{13}C NMR spectra were much lower than those determined by SEC. That is to say the overestimation effect of SEC measurements calibrated by polystyrene is probably more serious than underestimation for hyperbranched poly(ester-amide)s with numerous hydroxyl end groups.

Synthesis and Characterization of HBPEAs Based on Other Neutral α-Amino Acids

The previous results show that the "AD + CBB′" technique was successfully used to the monomer pair composed of 1-thioglycerol and D,L-alanine-based monomer to produce high level molecular weight HBPEAs with $Ti(OBu)_4$ as the efficient polymerization catalyst. Therefore, we tried extending this strategy to other neutral α-amino acids with different substituents at the α-position, such as glycine, D,L-2-aminobutyric acid, and D,L-phenylalanine, to investigate the steric effect of the intermediate on the polymerization and polymer properties. The ^1H and ^{13}C NMR, ESI-MS, HPLC, and elemental analyses were carried out at the first stage to ensure the formation of expected ABB′ intermediates with good yields. The same results were obtained as those based on D,L-alanine. The predicted ABB′ intermediates were firstly formed dominantly under the same mild conditions, and then *in situ* self-polycondensations were carried out at higher temperatures in the presence of $Ti(OBu)_4$. The polymerization conditions and results were illustrated in Table 2.

When compared with the polymerization of intermediate **3b** ($\mathbf{P_{b3}}$ in Table 2), crosslinking occurred more easily for intermediate **3a**, indicating a faster polymerization rate for the later, possibly because of its smaller steric hindrance. For intermediate **3a**, lower polymerization temperature should be adopted ($\mathbf{P_{a1}}$). In contrast, the molecular weights of the polymers $\mathbf{P_c}$ and $\mathbf{P_d}$ prepared from the intermediates **3c** and **3d,** respectively, at the same conditions to P_{b3}, were too low to be measured by SEC due to larger steric effects of ethylene and benzyl groups in the intermediates. For the intermediate **3c**, the polymers with molecular weight of 40.7 ($\mathbf{P_{c1}}$) and 40.0 kg/mol ($\mathbf{P_{c2}}$) were obtained, respectively, when the reaction time was prolonged to 19 h at 165 °C and the polymerization temperature was raised to 175 °C for 8.5 h. The steric effect on the polymerization was more obvious for intermediate **3d** possessing larger substituent. Polymer with molecular weight of 84.8 kg/mol was obtained when the reaction was proceeded for 26 h at 165 °C ($\mathbf{P_{d1}}$), and even 117.3 kg/mol when the temperature was increased to 180 °C for 12 h ($\mathbf{P_{d3}}$). However, temperatures higher than 180 °C should not be considered because serious side reactions and carbonization of the products occurred undesirably. The DP values evaluated by quantitative ^{13}C NMR spectra are showed in Table 2, indicating that the data determined by SEC measurements seriously deviate from that by quantitative ^{13}C NMR spectra which may be more credible. The DB values of the polymers based on glycine, D,L-2-aminobutyric acid, and D,L-phenylalanine were determined to be 0.56, 0.53, and 0.41 (Formula 1) and 0.53, 0.50, and 0.33 (Formula 2) on the basis of quantitative ^{13}C NMR, respectively. The PDIs of the polymers, in accordance with those of HBPEAs based on D,L-alanine, were close to 2.0 because of repeating purification process.

The thermal properties of the hyperbranched polymers obtained are also listed in Tables 1 and 2. The T_g of $\mathbf{P_{b3}}$ catalyzed by $Ti(OBu)_4$ is the highest in comparison with that of the polymers catalyzed by other three catalyst ($\mathbf{P_{b5}}$–$\mathbf{P_{b7}}$). This can be ascribed to the difference of their end group densities as a result of the different DBs of the polymers. The DB of $\mathbf{P_{b3}}$ is higher than those of $\mathbf{P_{b5}}$–$\mathbf{P_{b7}}$, which produces greater terminal group density and result in stronger intermolecular interaction of the H-bond. The results suggest that T_g slightly increases with the increase of DB for the hyperbranched poly(ester-amide)s with hydroxyl end groups although hardly influenced by the molecular weight of the polymer.[60] On the other hand, the higher T_g of the polymer derived from D,L-phenylalanine comparing those of polymers based on glycine, D,L-alanine, and D,L-2-aminobutyric acid may be attributed to hindered segmental rotation and greater rigidity as a result of the existence of the phenyl group, whereas the latter three contain more flexible alkyl

backbone. The temperatures of 10% mass loss ($T_d^{10\%}$) of the HBPEAs measured in air medium are commonly lower than those in nitrogen medium (see Supporting Information Fig. S5). This is possibly because a complicated set of chemical transformations was caused by exposure to high temperatures, which are a function of the environment. Thermooxidative processes take place in oxidizing medium (in air), thermal degradation predominant in neutral medium (in nitrogen or a vacuum), whereas pyrolysis takes place as high temperatures in neutral medium with formation of coke residue.[61] Among the various polymers, those based on D,L-phenylalanine display the highest thermal stability due to special structure. The $T_d^{10\%}$s are 289.1 °C in nitrogen and 265.6 °C in air for P_{d2}.

CONCLUSIONS

The "AD + CBB′" methodology have been successfully developed to prepare amino acid-based hyperbranched poly(ester-amide)s with numerous hydroxyl end groups in this study. Benefiting from merits of Michael addition reaction and higher nucleophilicity of mercapto than hydroxyl group, ABB′ intermediates were dominantly formed by thio-Michael addition of commercially available monomer 1-thioglycerol to the compounds prepared from neutral α-amino acids, which were demonstrated by ^{1}H and ^{13}C NMR, ESI-MS, HPLC, and elemental analyses. *In situ* self-condensations of these intermediates started at elevated temperature in the presence of a catalyst to produce HBPEAs with molecular weights range of 11.8–117.7 kg/mol with broad distribution determined by SEC measurements in contrast to 5.0–25.6 kg/mol estimated on the basis of quantitative ^{13}C NMR spectra. The influence factors of polymerization were investigated. $Ti(OBu)_4$ was found to be the most efficient catalyst compared with $SnO(Bu)_2$, Sb_2O_3, and $Zn(OAc)_2$. The propositional polymerization temperature was in the range of 160–180 °C. Structure of the intermediates significantly affected the molecular weights and properties of the corresponding HBPEAs, which originated from different steric hindrances on the α-carbon of the amino acids. For the HBPEAs obtained, the $T_d^{10\%}$s are above 220 °C, showing lower values in air medium than those in nitrogen, and glass transition temperatures (T_g) are in the range of −25.5 to 36.5 °C, depending on the structure of the monomers and slightly influenced by the DBs of the polymers bearing hydroxyl end groups. Among them, those derived from D,L-phenylalanine display the lowest degree of branching, the highest glass transition temperature and the best thermal stability. The large number of peripheral hydroxyl groups of these potential biodegradable and biodegradable HBPEAs will expand their range of applications.

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